2 GJE-59

#### Claim 4 (amended):

The [An] antibody, according to [any preceding claim] claim 1, which is non-rodent.

# Claim 5 (amended):

The [An] antibody, according to [any preceding claim] claim 1, which has affinity for a tumor-associated antigen

## Claim 6 (amended):

The [An] antibody, according to claim 5, wherein the antigen is carcinoembryonic antigen.

#### Claim 7 (amended):

The [An] antibody, according to [any preceding claim] claim 1, which is a single-chain Fv,  $F(ab')_2$ , Fv or fab.

#### Claim 8 (amended):

The [An] antibody, according to claim 7, having a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof having at least the same properties determined by the steps defined in claim 1.

### Claim 9 (amended):

A polynucleotide molecule encoding [an antibody according to claim 8,] <u>a high-affinity</u> monoclonal antibody, wherein the affinity of said antibody is characterisable by:

- (i) incubating first and second samples of the antibody in antigen-coated microtitre plate wells at a concentration chosen to be within the linear part of a standard curve at pH 7.2 for 1 hour at 37°C;
  - (ii) removing unbound antibody from both samples;
- (iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37°C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37°C;

3 GJE-59

- (iv) removing unbound antibody from both samples:
- (v) incubating both samples with anti-antibody alkaline phosphatase-conjugate for 1 hour at 37°C;
  - (vi) removing unbound conjugate from both samples; and
- (vii) adding PNPP substrate to the samples, measuring the absorbance of the samples at 405 nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is > 50% of that of the first sample,

wherein said antibody has a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof;

and wherein the polynucleotide comprises a nucleotide sequence defined in SEQ ID Nos. 1 and 3, or a variant thereof.

#### Claim 10 (amended):

A cloning vehicle comprising a polynucleotide molecule encoding a high-affinity monoclonal antibody, wherein the affinity of said antibody is characterisable by:

- (i) incubating first and second samples of the antibody in antigen-coated microtitre plate wells at a concentration chosen to be within the linear part of a standard curve at pH 7.2 for 1 hour at 37°C;
  - (ii) removing unbound antibody from both samples;
- (iii) incubating the first sample with PBS at pH 7.2 for 1 hour at 37°C, and reducing the pH of the second sample to pH 3 or below and incubating for 1 hour at 37°C;
  - (iv) removing unbound antibody from both samples;
- (v) incubating both samples with anti-antibody alkaline phosphatase-conjugate for 1 hour at 37°C;
  - (vi) removing unbound conjugate from both samples; and
- (vii) adding PNPP substrate to the samples, measuring the absorbance of the samples at 405 nm, and determining the amount of antibody bound to antigen, wherein the amount bound in the second sample is > 50% of that of the first sample;

wherein said antibody has a heavy chain variable region comprising the amino acid sequence defined in SEQ ID No. 2 and a light chain variable region comprising the amino acid sequence defined in SEQ ID No. 4, or a variant thereof;

<u>and wherein</u> the polynucleotide comprises a nucleotide sequence defined in SEQ ID Nos. 1 and 3, or a variant thereof.

The Commissioner is hereby authorized to charge any fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Respectfully submitted,

David R. Saliwanchik

Patent Attorney

Registration No. 31,794

Phone No.:

352-375-8100

Address:

2421 N.W. 41st Street

Suite A-1

Gainesville, FL 32606

DRS/la